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(54) Title: AIDS THERAPEUTICS BASED ON HIV-2 VPX PEPTIDES

(57) Abstract

Methods and therapeutic compositions for treating HIV-1 infection by administrating a Vpx polypeptide, particularly a Vpx polypeptide of HIV-2 or SIV.

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AIDS THERAPEUTICS BASED ON HIV-2 VPX PEPTIDES

Background of the Invention

This invention relates to the treatment of infection with the human immunodeficiency virus, type-1 (HIV-1, also denominated HTLV-III, LAV or ARV).

HIV-1 is an etiological agent of AIDS. This virus is generally described in Barre-Sinoussi et al., Science 220:868, 19831 Gallo et al., Science 224:500, 1984; Popovic et al., Science 224:497, 1984; and Levy et al., Science 225:840, 1984, each of which is hereby incorporated by reference. Various isolates of HIV-1 have been obtained from North America, Western Europe and Central Africa. These isolates differ somewhat in their nucleotide sequence, but the proteins they encode are generally antigenically cross-reactive.

A second virus related to HIV-1 has been isolated and termed HIV-2. This virus is reported by Guyader et al., Nature 326:662, 1987; Brun-Vezinet et al., The Lancet 1:128, 1987; and Clavel et al., Science 233:343, 1986, each of which is hereby incorporated by reference. Although the genetic organization of HIV-2 is similar to that of HIV-1, the two genomes cross-hybridize poorly even under low stringency conditions (Guyader et al., Nature 326:622, 1987).

A group of viruses isolated from monkeys, termed simian immunodeficiency virus (SIV or STLV-III), is related to HIV-1 and HIV-2, particularly the latter. See Daniel et al., Science 228:1201-1204 (1985); Kanki et al., Science 230:951-954 (1985); Chakrabarti et al., Nature 328:543-547 (1987); and Ohta et al., Int'l. J. Cancer 41:115-222 (1988), each of which is hereby incorporated by reference. Members of this viral group

exhibit minor variations in their genomic sequences, and have some differences in their restriction enzyme maps.

HIV-1 has already entered large segments of the world population, and substantial effort has been directed toward developing treatments for individuals infected with it. In addition to investigations into synthetic pharmaceuticals, effort has been directed toward utilizing variants of HIV-1 and HIV-2 to design AIDS therapeutics.

Intracellular immunization using *gag* gene mutants or capsid targeted Gag-nuclease fusion molecules have been described as potential anti-retroviral strategies (Trono et al., Cell 59:113-120 (1989); Natsoulis et al., Nature 352:632-635 (1991)).

Summary of the Invention

We have discovered that certain Vpx polypeptides encoded by the vpx gene from the SIV/HIV type-2 subgroup of viruses that are related to, but different from, HIV-1 (specifically HIV-2, SIV, and related viruses) exert an inhibitory effect on HIV-1 infection. We use the term Vpx polypeptides to describe these polypeptides, and, by that term, we mean to include the polypeptide encoded by the HIV-2 open reading frame termed orfX or vpx which has about 336 basepairs and is located in the central region of the HIV-2 genome between the pol ORF and the env ORF. See, e.g., Henderson et. al Science 241:199- 201 (1988); Yu et al. Nature 335:262- 265 (1988); Guyader et al. Nature 326: 662-669 (1987), each of which is hereby incorporated by reference. See also commonly owned USSN 07/179,758 which is hereby incorporated by reference. We include in that definition the products of the orfX open reading frames of all strains and variants of HIV-2, including LAV-2, SIV_{mac}, SIV_{smm}, and others (but not HIV-1 or its variants). We also include the orfX products of SIV and its variants. Moreover the spirit of the

invention encompasses the use of fragments and variants of Vpx polypeptides as described in Section I below.

Accordingly, the invention features a method of treating a patient infected with human immunodeficiency virus-type I (HIV-1) by administering a Vpx polypeptide in an amount effective to reduce pathogenic HIV-1 levels in the patient. Without wishing to bind ourselves to a specific mechanism, it appears that Vpx polypeptides curtail HIV-1 replication, thereby reducing overall viral load. The Vpx polypeptides may be delivered by various vehicles as described below.

In one embodiment, the Vpx polypeptide is administered to the patient in a pharmaceutically acceptable carrier and in an amount effective to reduce pathogenic HIV-1 levels in the patient. Alternatively, in a currently preferred embodiment, the patient is administered a therapeutic composition comprising nucleic acid encoding the Vpx polypeptide in an expressible genetic construction, e.g., one capable transforming patients' cells, such as a viral vector capable of infecting the patient. The vector may be administered directly to the patient, or cells may be removed from the patient and transformed with the above-described nucleic acid, after which the transformed cells are returned to the patient's body. Suitable viral vectors include HIV-1 and HIV-1 within a retroviral vector. Figure 4, below, gives the sequence of specific Vpx polypeptides.

The nucleic acid administered to the patient may also comprise a sequence encoding (or the VPX polypeptide may include) a CD4-binding polypeptide (such as a HIV-1 gp120) to facilitate targeting of HIV-1 infected cells expressing CD4 or a gp120-binding polypeptide (such as a CD4 polypeptide) to facilitate targeting to HIV-1 infected cells expressing gp120.

Another aspect of the invention features therapeutic compositions adapted for administration to a patient infected with human immunodeficiency virus-type I (HIV-1). The therapeutic compositions may include a Vpx polypeptide in a pharmaceutically acceptable carrier and at a dosage effective to reduce HIV-1 infection.

Alternatively, the invention features therapeutic compositions comprising nucleic acid encoding a Vpx polypeptide in an expressible genetic construction, such as any of those described above.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments of the invention and from the claims.

Description of the Preferred Embodiment(s)

The drawings will first be briefly described.

Drawings

Figure 1 is a diagram representing the construction of an HIV-1 viral vector containing vpx. A representative genomic organization of the HIV-1 and part of the HXB2UX and HXB2UΔX are shown.

Figure 2 is an immunoblot analysis of the sucrose gradient purified-HXB2UX and HXB2UΔX virions.

Figure 3 is a graph representing an infectivity study on a human CD4-positive T cell line (SupT1) with HXB2UX and HXB2UΔX viruses.

Figure 4 diagrams the primary structure of Vpx polypeptides.

Figure 5 retroviral vectors for the administration of the Vpx polypeptide by gene transfer therapy.

I. VPX Polypeptides

As described above, the invention includes therapies using any protein which is homologous to simian immunodeficiency virus/human immunodeficiency virus type-2 (SIV/HIV type 2) Vpx (Fig. 4, SEQ ID NOS: 1, 2 and 3)

as well as other naturally occurring Vpx polypeptides. Also included are: allelic variations; natural mutants; induced mutants; proteins encoded by DNA that hybridizes under high (e.g., washing at 2xSSC at 40 C with a probe length of at least 40 nucleotides) stringency conditions to naturally occurring Vpx encoding nucleic acid (for other definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference). The term also includes chimeric polypeptides that include Vpx together with unrelated sequences.

The invention also includes any biologically active fragment or analog of Vpx. By "biologically active" is meant possessing therapeutically useful anti-HIV-1 activity which is characteristic of the 112-amino acid Vpx polypeptides shown in Fig. 4 (SEQ ID NOS: 1, 2 and 3). Therapeutically useful activity of a Vpx fragment or Vpx analog, can be determined in any one (or more) of a variety of Vpx assays, for example, those assays described in this application. A Vpx analog possessing, most preferably 90%, preferably 40%, or at least 10% of the activity of 112-amino acid Vpx polypeptides (shown in Fig. 4; SEQ ID NOS: 1, 2 and 3), in any <u>in vivo</u> or <u>in vitro</u> Vpx assay (e.g., those described below), is considered biologically active and useful in the invention.

Preferred analogs include 112-amino acid Vpx (or biologically active fragments thereof) whose sequences differ from the wild-type sequence only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not destroy the polypeptide's relevant biological activity as measured

using in vivo or in vitro (e.g., those described above). Preferred analogs also include Vpx (or biologically active fragments thereof) which are modified for the purpose of increasing peptide stability; such analogs may contain, for example, one or more desaturated peptide bonds or D-amino acids in the peptide sequence.

Analogs can differ from naturally occurring Vpx by amino acid sequence differences or by modifications that do not affect sequence, or by both. Analogs of the invention will generally exhibit at least 65%, more preferably 80%, even more preferably 90%, and most preferably 95% or even 99%, homology with all or part of a naturally occurring Vpx sequence. The length of comparison sequences will generally be at least about 15 amino acid residues, preferably more than 40 amino acid residues. Modifications include *in vivo*, or *in vitro* chemical derivatization of polypeptides, e.g., acetylation, glycosylation, or carboxylation. Also embraced are versions of the same primary amino acid sequence that have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine. Analogs can differ from naturally occurring Vpx by alterations of their primary sequence. These include genetic variants, both natural and induced. Also included are analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Alternatively, increased stability may be conferred by cyclizing the peptide molecule.

In addition to substantially full-length polypeptides, the invention also includes biologically active fragments of the polypeptides. As used herein, the term "fragment", as applied to a polypeptide, will ordinarily be at least about 10 contiguous amino acids,

typically at least about 20 contiguous amino acids, more typically at least about 30 contiguous amino acids, usually at least about 40 contiguous amino acids, preferably at least about 50 contiguous amino acids, and most preferably at least about 60 to 80 or more contiguous amino acids in length. Fragments of Vpx can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of Vpx can be assessed by methods described below. Also included are Vpx polypeptides containing amino acids that are normally removed during protein processing (if any), including additional amino acids that are not required for the biological activity of the polypeptide (if any), or including additional amino acids (if any) that result from alternative mRNA splicing or alternative protein processing events.

The invention also includes polypeptides (or nucleic acid either encoding polypeptides) which are homologous to the Vpx protein or homologous to the vpx gene and are useful for the treatment of individuals infected with HIV-1. Sequences which are considered to be homologous are those which are 70 % homologous. Homologous refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences. For example, 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology.

II. Therapeutic Administration of Vpx Polypeptide.

With the availability of the cloned gene, the substantially pure Vpx polypeptide can be produced in quantity using standard techniques (Scopes, R. Protein Purification: Principles and Practice 1982 Springer-Verlag, NY). Thus, another aspect of the invention is a pharmaceutical comprising the Vpx polypeptide together with an acceptable diluent, carrier or excipient and/or in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the polypeptide to patients infected with HIV-1.

A substantially pure preparation of a polypeptide is a preparation which is substantially free (e.g., to the extent required for formulating Vpx into a therapeutic composition) of the proteins with which it naturally occurs in a cell.

Fragments or analogs of the Vpx protein may also be administered to a patient infected with HIV-1 in the manner described above. Fragments or analogs which are useful for this purpose include those which are described above and are useful for the treatment of a patient infected with HIV-1. Fragments and analogs which will be useful for the therapeutic treatment of patients infected with HIV-1 are determined using the assays provided in the examples, below, among others.

The Vpx polypeptide may also be administered to a patient infected with HIV-1 in the form of a fusion protein consisting of a Vpx polypeptide, fused to the gp120 protein, or a fragment thereof which is sufficient to bind the CD4 receptor of T cells. This fusion protein allows delivery of the Vpx polypeptide into uninfected T cells expressing the CD4 receptor.

The Vpx polypeptide may also be administered to a patient infected with HIV-1 in the form of a fusion protein consisting of the Vpx polypeptide, or a therapeutically useful fragment or derivative, fused to the CD4 protein, or a fragment thereof, which is sufficient to bind gp120. This fusion protein allows delivery of the Vpx polypeptide into infected T cells expressing gp120 on their surface. The Vpx-gp120 fusion polypeptide or the Vpx-CD4 fusion polypeptide may be generated using standard techniques of molecular biology to generate fusions encoded from a suitable vector (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1989)). Either the gp120 fragment or the CD4 fragment may enable internalization of the Vpx polypeptide through endocytosis. The usefulness of such gene fusions constructs may be determined using the methods described below in the examples, among others. The invention includes administering either fusion polypeptide alone in a pharmaceutically acceptable carrier, or administering both fusions together in an acceptable carrier.

Thus, the formulations of this invention can be applied for example by parenteral administration, intravenous, subcutaneus, intramuscular, intracranial, intraorbital, opthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration.

Therapeutic Formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are to be found in, for example, "Remington's Pharmaceutical Sciences". Formulations for

parenteral administration may, for example, contain excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of present factors. Other potentially useful parenteral delivery systems for the factors include ethylene-vinyl acetate copolymer particals, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally.

III. Construction of HIV-1 Containing Vpx

A particularly preferred embodiment features administering to the patient genetic constructions which encode any of the above-described Vpx polypeptides, and (after transformation of patient cells) can express the Vpx polypeptide.

In addition to the HIV-1 example below illustrating a preferred viral vehicle, those skilled in the art will readily appreciate that the invention can use other HIV strains of the many that have been fully characterized e.g., MN, HXB2, LAI, NL43, MFA, BRVA and z321.

Moreover, there are numerous other viral vehicles (i.e., nucleic acid vehicles which can activate or be activated to enter cells of the host organism and, having done so, to be expressed there.

Examples

The following examples are provided to illustrate the invention not to limit it.

Example I

A therapeutic, using Vpx of SIVmac is illustrated below. Any of the above described Vpx polypeptides can be incorporated into an anti-HIV-1 therapeutic by the same technique. Vpx has been shown to bind to nucleic acids and may also play a structural role as suggested by its abundance in the virions (Henderson et al., Science 241:199-201 (1988). To express vpx from an HIV-1 genome, HXB2UX was generated (Fig.1). HXB2UX is a derivative of an infectious molecular clone of HIV-1, HXB2 (Ratner et al., AIDS Res. Hum. Retroviruses 3:57-69 (1987)). This expression utilizes Vpx of SIVmac (Kornfeld et al., Nature 326:610-613 (1987)) in cis to analyze its effects on HIV-1 replication during multiple rounds of infection because the vpx then co-segregates with the HIV-1 genome. As a control, HXB2UΔX, an isogenic clone of HXB2UX except for a premature stop codon in vpx, was prepared.

A representative genomic organization of the HIV-1 and part of the HXB2UX and HXB2UΔX are shown in Figure 1. For both constructs, the parental clone was the infectious molecular HIV-1 clone HXB2 (Ratner et al., AIDS Res. Hum. Retroviruses 3:57-69 (1987). HXB2UX contains vpx derived from SIVmac (BK28) (Kornfeld et al., Nature 326:610-613 (1987). HXB2UΔX contains vpx with a premature stop codon introduced by an Xba I linker after the first twenty amino acids of Vpx (Yu et al., Nature 335:262-265 (1988)). Unlike HXB2, both clones have an intact vpu initiation codon. The restriction enzymes used for the cloning are also shown.

All the mutations were done by the method of T.A. Kunkel (Proc. Natl. Acad. Sci. USA 82:488-492 (1985)). A Cla I site was introduced at the beginning of *vpr* with a

mutagenic oligonucleotide 5′-GGG CTT GTT CCA TCG ATT CTC TGT CAG TTT C-3′ (SEQ ID NO: 4). The template for the mutagenesis and the cloning strategy were previously described (Yuan et al., AIDS Res. Hum. Retroviruses 6:1265-1271 (1990)). DNA fragments of vpx or the vpx with a premature stop codon were prepared by polymerase chain reaction (PCR) (Saiki et al., Science 239:487-491 (1988)) with two primers 5′-TAA AAG TAG TAA TCG ATG TCA GAT CCC AGG GAG-3′ (SEQ ID NO: 5) and 5′-GCG GGG GTC GAC TTA TGC TAG TCC TGG AGG GGG-3′ (SEQ ID NO: 6). Each contained restriction sites (Cla I and Sal I, respectively) needed for cloning. The template for the PCR was pBK28 for vpx and pBK28ΔX for vpx with a premature stop codon, respectively, and the PCR condition was as suggested by the manufacturer. pBK28 and pBK28ΔX were previously described (Yu et al., Nature 335:262-265 (1988)). Each PCR product was cloned into HXB2U, a clone isogenic to HXB2 (Ratner et al., AIDS Res. Hum. Retroviruses 3:57-69 (1987)) except for an intact vpu initiation codon, as a ClaI-SalI fragment. By this cloning, the original *vpr* was destroyed. To reconstruct the *vif* truncated by this procedure, two oligonucleotides 5′-CGC TGG AAC AAG CCG CAG AAG ACG AAG GGC CAT CGC GGC AGC CAC ACG ATC AAC GGA CAC TAG TCA CCA T-3′ (SEQ ID NO: 7), 5′-CGA TGG TGA CTA GTG TCC GTT GAT CGT GTG GCT GCC GCG ATG GCC CTT CGT CTT CTG CGG CTT GTT CCA G-3′ (SEQ ID NO: 8) were synthesized and annealed and then cloned into the Cla I site. The resulting clones HXB2UX and HXB2UΔX were verified by DNA sequencing (Sanger and Coulson, J. Mol. Biol. 94:441-445 (1975)).

Both HXB2UX and HXB2UΔX constructs were transfected into Cos-7 cells and the released viruses were purified through a sucrose gradient and analyzed by immunoblotting with a reference serum from an HIV-1 infected individual and a goat serum specific for Vpx

(Fig. 2). Human serum revealed an almost identical protein profile for both virions. The *gag* products, p17 and p24; and the *pol* products, p66 and p51, were readily detected (Fig. 2, lanes 1 and 2). When probed with the goat anti-Vpx serum, a protein of relative molecular mass of about 12,000 (M_r12K) was detected in HXB2UX virions (Fig. 2, lane 4), but was not observed in HXB2UΔX virions (Fig. 2, lane 3). The size of the protein found in HXB2UX corresponded with that of the Vpx described previously (Yu et al., Nature 335:262-265 (1988)). These data showed that Vpx expressed in the context of an HIV-1 genome, was packaged into cell-free HIV-1 virions.

The immunoblot analysis of the sucrose gradient purified-HXB2UX and HXB2UΔX virions is now described in detail. The duplicate blots prepared from the same protein gel were used for immunoblot analysis. Immunoblots were probed with a representative serum for an HIV-1 infected individual (Figure 2, lanes 1, 2). Position of the *gag* product p17 and p24; the *pol* product p66 and p51 were indicated. The immunoblot probed with the goat anti-Vpx serum (Figure 2, lanes 3, 4). The position of the Vpx is indicated by an arrow. The protein of relative molecular mass of about 26,000 (M_r26K) observed in Figure 2, lane 4 might represent a dimeric form of Vpx. Standard relative molecular mass markers for proteins are indicated on the left in Kd.

METHOD: Proviral DNA HXB2UX and HXB2UΔX were transfected into Cos-7 cells by the DEAE-dextran method and at 72 hours post-transfection viruses were collected and purified by a sucrose density gradient was described previously (Yuan et al., AIDS Res. Hum. Retroviruses 6:1265-1271 (1990)). Aliquots of each fraction were used to measure RT activity (Rhol et al., Virology 112:355-360 (1981)) and the viruses derived from the two fractions at RT peaks were collected by ultracentrifuge and used for

the immunoblot analysis. The procedure of the immunoblot was described before (Barin et al., Lancet ii, 1387-1389 (1985)).

Example 2

To examine the effects of the incorporated Vpx on viral infectivity, HXB2UX and HXB2UΔ viruses derived from the transfected Cos-7 cells were used to infect a CD4-positive human T cell line, SupT1, which is susceptible to HIV-1 infection. The infection was monitored by reverse transcriptase (RT) activity of the culture supernatant. A representative result is shown in Fig. 3. RT activity was detected in HXB2UΔX infected SupT1 at day 15 and continued to increase. In contrast, the RT activity of HXB2UX infected SupT1 was similar to an uninfected control throughout the observation period of 34 days. Consistent with this RT data, cytopathic effects were readily detected in HXB2UΔX infected cells whereas no obvious cytopathic effects were observed in HXB2UX infected cells.

Representative infectivity studies on human CD4-positive T cell line SupT1 with HXB2UX and HXB2UΔX viruses are described here in detail and shown in Figure 3. The viruses produced from the transfected Cos-7 cells were used to infect SupT1 cells and the RT activity released into the culture supernatant was determined at the time points indicated. HXB2UX (open square), HXB2UΔX (closed circle), and Mock (open circle) infected SupT1 cells.

METHODS. Proviral DNA were transfected into Cos-7 cells, and 72 hours after transfection the viruses were collected and used for the infection. The input virus dose was adjusted by the measured RT activity. Cells were incubated with the corresponding viruses at 37°C about 17 hours and washed. They were then maintained in fresh culture medium. The sample preparation and

measurements of the RT were as described before (Rho et al., Virology 112:355-360 (1981)).

IV. Therapeutic Administration of Vpx in a Viral Vector.

Retroviral vectors, or other viral vectors with the appropriate tropisms for cells infected by HIV-1, may be used as a gene transfer delivery system for the Vpx polypeptide. Numerous vectors useful for this purpose are generally known have been described (Miller, Human Gene Therapy 15-14 (1990); Friedman, Science 244:1275-1281 (1989); Eglitis and Anderson, BioTechniques 6:608-614 (1988); Tolstoshev and Anderson, Current Opinion in Biotechnology 1:55-61 (1990); Sharp, The Lancet 337:1277-1278 (1991); Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322 (1987); Anderson, Science 226:401-409 (1984); Moen, Blood Cells 17:407-416 (1991); and Miller and Rosman, Biotechniques 7:980-990 (1989)). Retroviral vectors are particularly well developed and have been used in a clinical setting (Rosenberg, et al N. Engl. J. Med 323:370 (1990)).

The retroviral constructs, packaging cell lines and delivery systems which may be useful for this purpose include, but are not limited to, one, or a combination of, the following: Moloney murine leukemia viral vector types; self inactivating vectors; double copy vectors; selection marker vectors; and suicide mechanism vectors. The Moloney murine leukemia retroviral system of Vpx delivery is particularly useful since it targets delivery of the Vpx protein to the hematopoietic cells which ultimately give rise to the T-cells. The delivery of the Vpx polypeptide can be further restricted to cells which are infected by HIV-1 directly by virtue of utilizing retroviral constructs in which the HIV-LTR is used to drive expression from the vpx gene. To achieve proper

expression from such a construct the 3'LTR of the Moloney murine leukemia vector must be deleted. Vector strategies which include either the entire HXB2UX construct or the vpx gene driven by the HIV-LTR are shown in Figure 5.

Fragments or derivatives of the Vpx polypeptide may also be administered by retroviral gene transfer therapy or another suitable viral vector system. Fragments or derivatives are defined as described above. Useful fragments or derivatives of Vpx may be administered by inserting the nucleic acids encoding these fragments or derivatives in place of the complete vpx gene in a gene therapy vector, as described above. Such constructs may be tested using the methods for testing the effects of Vpx on viral infectivity described above, among others.

Retroviral delivery of Vpx is particularly appropriate in HIV-1 infected individuals who display the common secondary appearance of B-cell tumors as a result of immunodeficiency. These individuals may undergo bone marrow removal, treatment, and reimplantation as a matter of course for the treatment of the B-cell tumors. At this time standard techniques for the delivery of gene therapy vectors may be used to transfect stem cells. Such transfection may result in Vpx synthesizing T-cells useful in lowering the infective levels of HIV-1 in the patient.

Non viral methods for the therapeutic delivery of nucleic acid encoding Vpx

Nucleic acid encoding Vpx, or a fragment thereof, under the regulation of the HIV-LTR and including the appropriate sequences required for insertion into genomic DNA of the patient, or autonomous replication, may be administered to the patient using the following gene transfer techniques: microinjection (Wolff et al.,

Science 247:1465 (1990)); calcium phosphate transfer (Graham and Van der Eb, Virology 52:456 (1973); Wigler et al., Cell 14:725 (1978); Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413 (1987)); lipofection (Felgner et al., Proc. Natl. Acac Sci. USA 84:7413 (1987); Ono et al., Neuroscience Lett 117:259 (1990); Brigham et al., Am. J. Med. Sci. 298:278 (1989); Staubinger and Papahadjopoulos, Meth. Enz. 101:512 (1983)); asialorosonucoid-polylysine conjugation (Wu and Wu, J. Biol. Chem. 263:14621 (1988); Wu et al., J. Biol. Chem. 264:16985 (1989)); and electroporation (Neumnn et al., EMBO J. 7:841 (1980)). These references are hereby incorporated by reference.

V. Mode of Vpx Action

As a potential mechanism for the observed interference in HIV-1 replication, while not essential to practicing the invention, is discussed below, with reference to the structural and non-structural effects of the Vpx protein can be considered. The targeting of Vpx, which is foreign to HIV-1 virions, may affect the assembly and/or maturation of HIV-1. Alternatively, the affinity of Vpx for single-stranded nucleic acids may be important for the interference of HIV-1 replication (Henderson et al., Science 241:199-201 (1988)). The data shown above demonstrate that Vpx expressed in the context of an HIV-1 genome was incorporated into HIV-1 virions and the resulting viruses lost infectivity in T cells. Therefore Vpx can be regarded as a virion-specific inhibitory molecule against HIV-1.

Previous studies showed the region near the carboxyl terminus was important for the function of Vpr of HIV-1 (Yuan et al., AIDS Res. Hum. Retroviruses 6:1265-1271 (1990); Cohen et al., J. AIDS, 3:11-18

(1990)). In Vpx, seven consecutive prolines are found at the carboxyl terminus which are absent in Vpx.

Both Vpx, which is only present in the SIV/HIV-2 group of lentiviruses, and Vpr, which is present in most of the SIV/HIV-2 group and the HIV-1 group, are virion-associated proteins. Some regions are highly conserved between Vpx and Vpr at the primary structure level (Fig. 4).

Figure 4 shows the primary structure of three Vpx polypeptides. Several Vprs and Vpxs were compared using a multiple sequence alignment program (GeneWorks, IntelliGenetics, California, USA). The sequences used were obtained from the Human Retroviruses and AIDS Database (Myers et al., Human Retroviruses and AIDS (Los Alamos National Laboratory, Los Alamos, USA 1990)) except for HIV-1 (Yuan et al., AIDS Res. Hum. Retroviruses 6:1265-1271 (1990)). The following isolates were analyzed: $HIV\text{-}2_{ROD}$, $SIVsmm_{H4}$ and $SIVmac_{MMZS1}$.

We infer that the potential inhibitory domain or domains of Vpx should reside in the regions that are non-homologous to HIV-1 Vpr, mainly the region near the C-terminus and in the central region. If this is the case, it suggests that a molecule for specifically inhibiting a virus need not be a fusion of a virion associated motif to a functioning domain such as a nuclease or protease thus allowing greater freedom to design such therapeutics.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Myron E. Essex et al.

(ii) TITLE OF INVENTION: AIDS Therapeutics Based on HIV-2 VPX Peptides

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Freeman, John W.
(B) REGISTRATION NUMBER: 29,066
(C) REFERENCE/DOCKET NUMBER: 00379/017001

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617/542-5070
(B) TELEFAX: 617/542-8906

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Ser Asp Pro Arg Glu Arg Ile Pro Pro Gly Asn Ser Gly Glu Glu
1 5 10 15

Thr Ile Gly Glu Ala Phe Glu Trp Leu Asn Arg Thr Val Glu Glu Ile
20 25 30

Asn Arg Glu Ala Val Asn His Leu Pro Arg Glu Leu Ile Phe Gln Val
35 40 45

Trp Gln Arg Ser Trp Glu Tyr Trp His Asp Glu Gln Gly Met Ser Gln
50 55 60

Ser Tyr Val Lys Tyr Arg Tyr Leu Cys Leu Met Gln Lys Ala Leu Phe
65 70 75

Met His Cys Lys Lys Gly Cys Arg Cys Leu Gly Glu Gly His Gly Ala
80 85 90

Gly Gly Trp Arg Pro Gly Pro Pro Pro Pro Pro Pro Pro Gly Leu Ala
95 100 105

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Asp Pro Arg Glu Arg Ile Pro Pro Gly Asn Ser Gly Glu Glu
1 5 10 15

Thr Ile Gly Lys Ala Phe Glu Trp Leu Asn Arg Thr Val Glu Glu Ile
20 25 30

Asn Arg Ala Ala Val Asn His Leu Pro Arg Glu Leu Ile Phe Gln Val
35 40 45

Trp Arg Arg Ser Trp Glu Tyr Trp His Asp Glu Met Gly Met Ser Glu
50 55 60

Ser Tyr Thr Lys Tyr Arg Tyr Leu Cys Leu Ile Gln Lys Ala Leu Phe
65 70 75

Val His Cys Lys Lys Gly Cys Arg Cys Leu Gly Glu Glu His Gly Ala
80 85 90

Gly Gly Trp Arg Thr Gly Pro Pro Pro Pro Pro Pro Gly Leu Ala
95 100 105

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Thr Asp Pro Arg Glu Thr Val Pro Pro Gly Asn Ser Gly Glu Glu
1 5 10 15

Thr Ile Gly Glu Ala Phe Ala Trp Leu Asn Arg Thr Val Glu Ala Ile
20 25 30

Asn Arg Glu Ala Val Asn His Leu Pro Arg Glu Leu Ile Phe Gln Val
35 40 45

Trp Gln Arg Ser Trp Arg Tyr Trp His Asp Glu Gln Gly Met Ser Glu
50 55 60

Ser Tyr Thr Lys Tyr Arg Tyr Leu Cys Ile Ile Gln Lys Ala Val Tyr
65 70 75

Met His Val Arg Lys Gly Cys Thr Cys Leu Gly Arg Gly His Gly Pro
80 85 90

Gly Gly Trp Arg Pro Gly Pro Pro Pro Pro Pro Pro Gly Leu Val
95 100 105

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGCTTGTTC CATCGATTCT CTGTCAGTTT C 31

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TAAAAGTAGT AATCGATGTC AGATCCCAGG GAG 33

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCGGGGGTCG ACTTATGCTA GTCCTGGAGG GGG 33

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CGCTGGAACA AGCCGCAGAA GACGAAGGGC CATCGCGGCA GCCACACGAT CAACGGACAC 60

TAGTCACCAT 70

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGATGGTGAC TAGTGTCCGT TGATCGTGTG GCTGCCGCGA TGGCCCTTCG TCTTCTGCGG 60

CTTGTTCCAG 70

CLAIMS

1. A method of treating a patient infected with human immunodeficiency virus-type I (HIV-1) by administering to said patient a Vpx polypeptide in an amount effective to reduce infective HIV-1 levels in said patient.

2. The method of claim 1 comprising administering to said patient a therapeutic composition comprising said Vpx polypeptide in a pharmaceutically acceptable carrier.

3. The method of claim 1 comprising administering to said patient a therapeutic composition comprising nucleic acid encoding said Vpx polypeptide in an expressible genetic construction.

4. The method of claim 1 comprising administering to said patient a therapeutic composition comprising nucleic acid encoding said Vpx polypeptide in an expressible genetic construction, capable of transforming cells of said patient.

5. The method of claim 4 in which said nucleic acid is part of a viral vector capable of infecting said patient.

6. The method of claim 3 in which said nucleic acid further comprises a sequence encoding a CD4-binding polypeptide.

7. The method in claim 3 in which the said nucleic acid further comprises a sequence encoding a gp120-binding polypeptide.

8. The method of claim 4, claim 5, claim 6, or claim 7 comprising removing cells from said patient, transforming said cells with said nucleic acid, and returning transformed cells to said patient's body.

9. The method of claim 4, claim 5, claim 6 or claim 7 comprising administering said said genetic construction directly to said patient.

10. The method of claim 5, wherein said viral vector is human immunodeficiency virus-type I.

11. The method of claim 1 or claim 2 wherein said Vpx polypeptide comprises the sequence of figure 4.

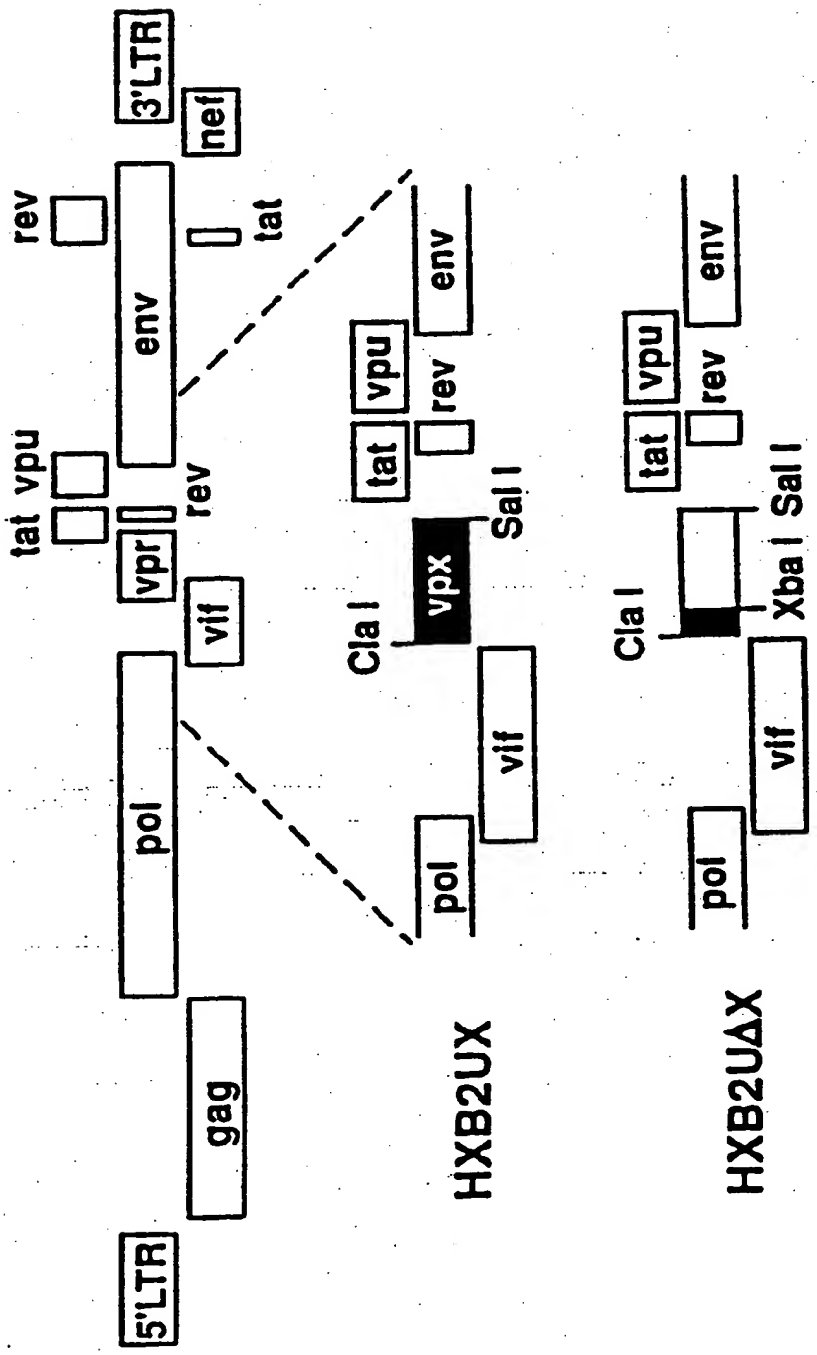
12. A therapeutic composition adapted for administration to a patient infected with human immunodeficiency virus-type I (HIV-1), said composition comprising a Vpx polypeptide in a pharmaceutically acceptable carrier.

13. A therapeutic composition adapted for administration to a patient infected with human immunodeficiency virus-type I (HIV-1), said composition comprising nucleic acid encoding a Vpx polypeptide in an expressible genetic construction for transforming cells of a human patient.

14. The therapeutic composition of claim 13 comprising said nucleic acid as part of a viral vector capable of infecting said patient.

15. The therapeutic composition of claim 13 wherein said nucleic acid further comprises a sequence capable of encoding a CD4-binding polypeptide.

16. The therapeutic composition of claim 13 wherein said nucleic acid further comprises a sequence encoding a gp120-binding polypeptide.


5'LTR
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vpu
env
rev
tat
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3'LTR
HXB2UX
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Cla I
vpx
Sal I
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Xba I
Sal I
tat
vpu
rev
env

FIG. 1

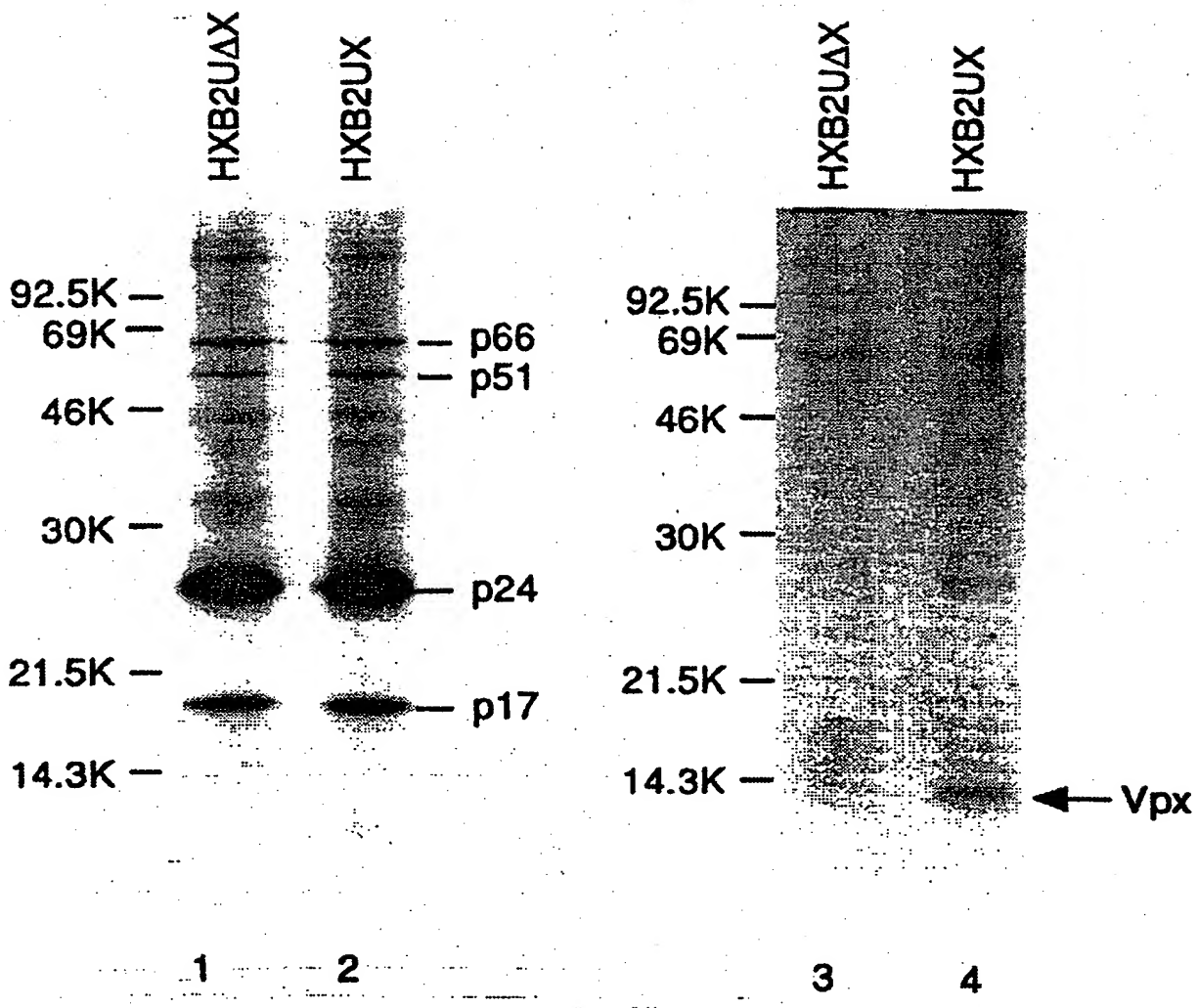

FIG. 2

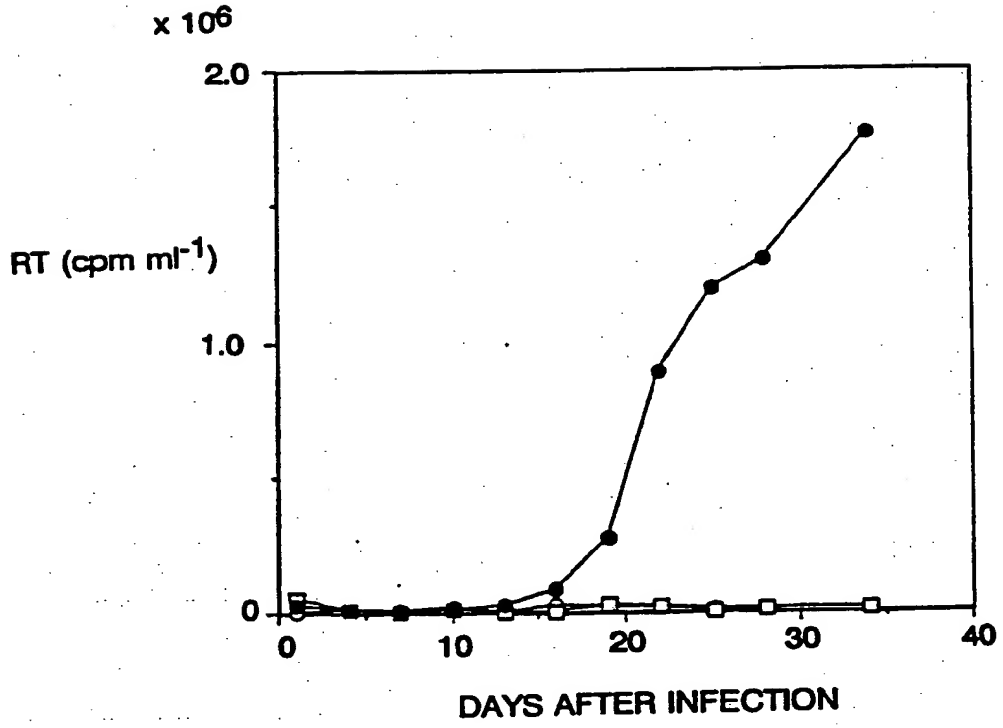

FIG. 3

```
SIVmacVpx  MSDPRERIPP GNSGEETIGE AFEWLNRTVE EINREAVNHL PRELIFQVWQ RSWEYWHDEQ 60
SIVsmmVpx  MSDPRERIPP GNSGEETIGK AFEWLNRTVE EINRAAVNHL PRELIFQVWR RSWEYWHDEM 60
HIV-2Vpx   MTDPRETVPP GNSGEETIGE AFAWLNRTVE EINREAVNHL PRELIFQVWQ RSWRYWHDEQ 60

SIVmacVpx  GMSQSYVKYR YLCLMQKALF MHCKKGCRCL GEGHGAGGWR TGPHPPPHPG LA 112
SIVsmmVpx  GMSESYTKYR YLCLIQKALF VHCKKGCRCL GEEHGAGGWR TGPHPPPHPG LA 112
HIV-2Vpx   GMSESYTKYR YLCLIQKAVY MHVKKGCTCL GRGHGPGGWE TGPHPPPHPG LV 112
```

FIG. 4

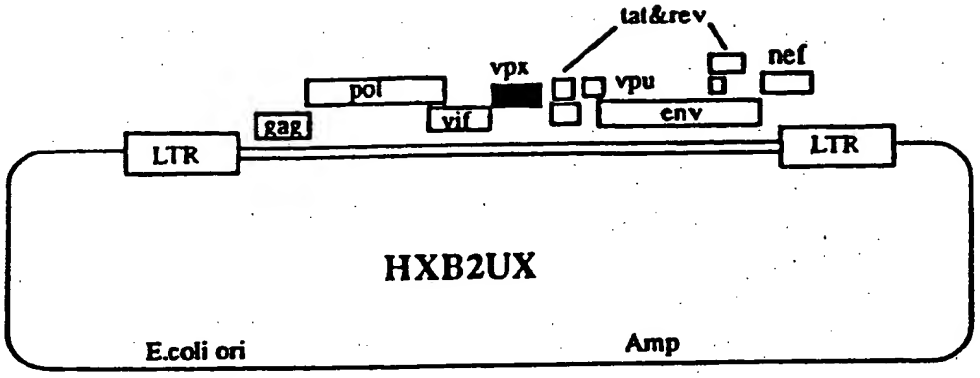

FIG. 5A

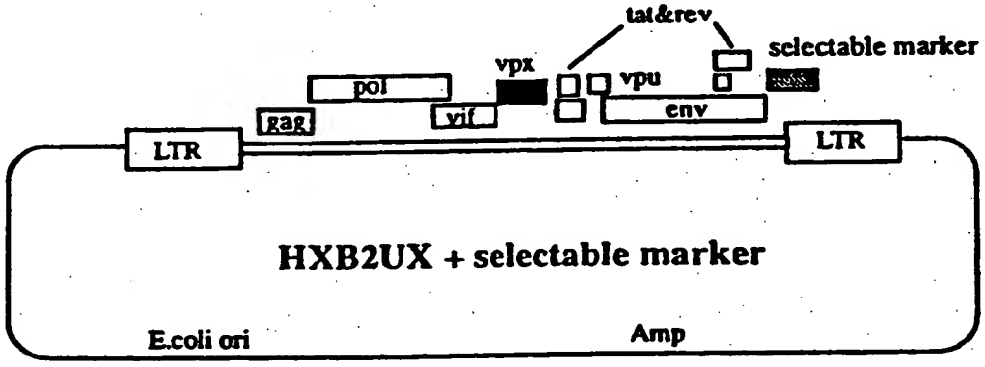

FIG. 5B

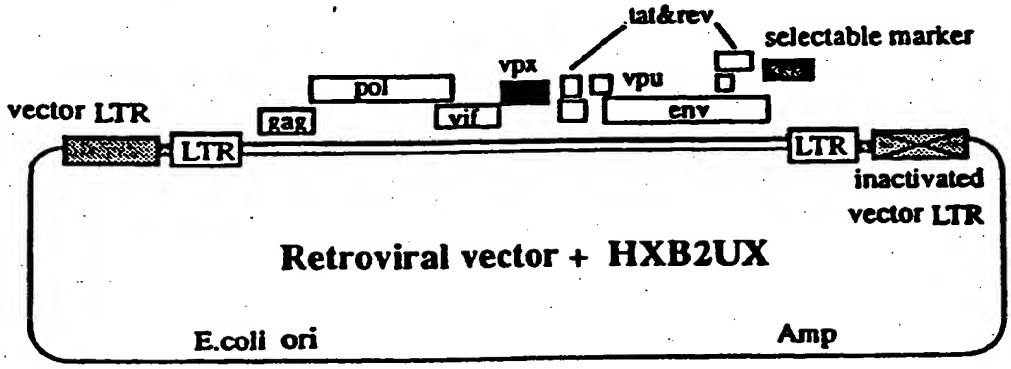

FIG. 5C

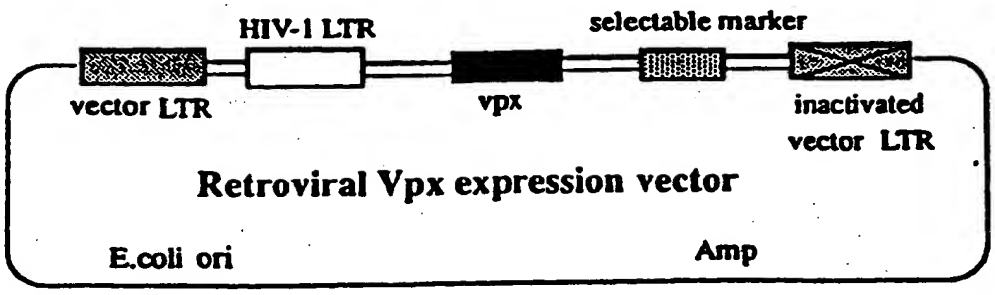

FIG. 5D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04301

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/21; C07K 13/00
US CL :424/88, 89; 530/350, 826; 514/885
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 530/350, 826; 514/885

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, MEDLINE, APS, AIDSLINE, BIOSIS, EMBASE, IG SUITE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Virology, Volume 65(7), issued July 1991, L. Marcon et al, "Dispensable Role of the Human Immunodeficiency Virus Type 2 Vpx protein in Viral Replication", pages 3938-3942.	1-16
A	Journal of Virology, Volume 65(9), issued September 1991, X.F. Yu et al, "The *vpx* Gene of Simian Immunodeficiency Virus Facilitates Efficient Viral Replication in Fresh Lymphocytes and Macrophages", pages 5088-5091.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
09 July 1993

Date of mailing of the international search report
14 JUL 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04301

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Virology, Volume 177(1), issued July 1990, A.R. Hart et al, "Interference Patterns of Human Immunodeficiency Viruses HIV-1 and HIV-2", pages 1-10.	1-16
A	Virology, Volume 184(1), issued September 1991, J.C. Kappes et al, "Human Immunodeficiency Virus Type 2 Vpx protein Augments Viral Infectivity", pages 197-209.	1-16
A,P	The EMBO Journal, Volume 11(9), issued September 1992, M. Tristem et al, "Evolution of the Primate Lentiviruses: Evidence from *vpx* and *vpr*", pages 3405-3412.	1-16
A	Proceedings of the National Academy of Sciences USA, Volume 89, issued January 1992, M. Le Guern et al, "Human Immunodeficiency Virus (HIV) Type 1 Can Superinfect HIV-2-Infected Cells: Pseudotype Virions Produced With Expanded Cellular Host Range", pages 363-367.	1-16